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Two areas where plant systems show promise for immediate use are: 1) in short-term bioassays used in the laboratory for mutagenicity evaluation of specific chemicals or chemical mixtures, and 2) as indicators of mutagenicity of the total environment under actual field conditions. This review focuses on the advantages of plants for mutation monitoring and dexcribes several model plant test systems.

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MONITORING FOR MUTAGENICITY WITH PLANTS

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INTRODUCTION

Recent advances in the development and use of short-term bioassays have shown the presence of mutagens and presumptive carcinogens in many pesticides, drugs, cosmetics, food additives, industrial products and by-products, and waste material from energy production and utilization processes. Growing evidence suggests that environmental chemicals may contribute to cancer, birth defects, and cardiovascular abnormalities.

Approximately 63,000 individual chemicals are known to be in commercial use in the United States (Maugh, 1978). Only a small fraction of these chemicals has been adequately tested for health and ecological effects. The potential health hazard to man from exposure to these chemicals is growing because approximately 500 new chemicals are synthesized and introduced into the environment every year. The major factors that have contributed to the delay in investigating the safety of these chemicals are the high cost and long study period associated with conventional testing employing animal species.

To expedite the evaluation of environmental substances, a number of short-term bioassays utilizing a variety of test organisms has been developed. These bioassays offer the advantages of being simple, rapid, and cost-effective. Most of these bioassays use *in vitro* methodologies and were developed to measure specific biological effects from treatment with individual chemicals. However, the spectrum of biological effects produced

by individual chemicals tested in the laboratory may not be the same when these chemicals are components of complex mixtures in the ambient environment. Therefore, the development and validation of simple in vivo bioassays employed under field conditions seem desirable.

Although plants have historically been important in the development of genetic principles and the illustration of the hazards of ionizing radiation, plants have not been adequately utilized when evaluating the mutagenic or presumptive carcinogenic effects of environmental chemicals. For testing under field conditions (in situ monitoring), few test organisms offer the advantages provided by plants. In view of the plant's ability to metabolically activate several classes of promutagens and because of the plant's role in our food chain, plant systems warrant consideration in environmental mutagenesis testing.

For the last five years, the U.S. Environmental Protection Agency (EPA) and the National Institute of Environmental Health Sciences (NIEHS) have made concerted efforts to develop and utilize plant bioassays. The current state-of-the-art on utility of plant bioassays for environmental monitoring has been published in Environmental Health Perspectives Volumes 27 (1978) and 37 (1981). A series of review papers on the degree of validation of a number of plant test systems for mutagenicity testing will be published shortly in Mutation Research as a product of the EPA GENE-TOX Program (Green and Auletta, 1981).

Two areas where plant systems show promise for immediate use are: 1) in short-term bioassays used in the laboratory for mutagenicity evaluation of specific chemicals or chemical mixtures, and 2) as indicators of mutagenicity of the total environment under actual field conditions. This review focuses on the advantages of plants for mutation monitoring and describes several model plant test systems. The test systems reviewed here have been selected on the basis of the degree of validation and definition of genetic end points for these bioassays. A number of other plant bioassays are also available for mutation monitoring, and the reader is referred to two recent reviews on this subject by Grant (1981) and Grant and Zura (in press).

THE ADVANTAGES OF PLANTS

Organization

Green plants, like animals, are eukaryotes; they contain similar amounts of DNA per cell and have a similar amount of

repetitive DNA as do animals. In addition, the regulation of gene action relative to ontogeny and the phenomena of competence and cell heredity are typical of higher organisms (Constantin, 1978; Freeling, 1978). Although animal cells lack a cell wall and plant cells lack centrioles, the chromosome organization of plants and animals, including the genome organization, is very much alike. However, because the chromosomes of certain plant species are large-sized and few in number, they are especially suitable for cytogenetic studies.

The plant characteristics of of polyploidy, diploidy, and haploidy, as well as self-fertilization and vegetative propagation -- rare in the animal kingdom, offer unique opportunities to uncover mutant genes. Many species complete their life cycle in less than a year, affording quick assays, although cells may sometimes be regenerated and cultured over long periods of time, enabling long-term chronic studies of pollutants.

Metabolism

In the area of metabolism, perhaps less is known about plant and animal similarities and differences. Protein synthesis appears to be similar in both groups, and the metabolic machinery for biotransformation (Menn, 1978) and metabolic activation (Nilan, 1978) is also at least qualitatively similar. While liver homogenate from mammals was once thought to be a prerequisite for activation of many promutagens into mutagens, it is now recognized that several plant species can make this conversion (Plewa and Gentile, 1981). For example, soybeans will activate nitrosamine and dimethylnitrosamine with exposure to chemical concentrations as low as 1.25 ppm for 24 h (Vig, 1978). For 56 test compounds representing a variety of chemical classes, Redei et al. (1980) found a correlation of 88% between the mutagenicity in *Arabidopsis* and carcinogenicity in laboratory animals. This finding, if widely applicable, indicates considerable potential for using plants in a tier approach as monitors of mutagens for man.

Recognizing that the rates of biotransformation and metabolic activation may differ greatly in plants and animals is important. Also, transport and excretion play important roles when evaluating the quantitative effects of these processes. Plants, lacking the efficient excretory machinery of animals, tend to be "integrators" of particulates, especially dusts, heavy metals, and aerosols (Feder, 1978). They can also easily absorb lipophilic substances, including many pesticides (Menn, 1978). In plants, many chemicals are retained for long periods of time and can be activated into mutagens. In animals, however, these chemicals are often excreted

rapidly. Thus, one would suspect that plants act as rather conservative test systems, affording more sensitive measurements of mutagen potential than animal test systems. This sensitivity in plant test systems could be regarded as an asset, since we are frequently interested in the effects of chronic, long-term exposures to man -- effects that may only be detectable in short-term tests with a collector-type organism.

Genetic End Points

A wide array of genetic end points including cytoplasmic mutations, paramutation, gene mutations, single-strand DNA breaks, DNA repair, and chromosome changes such as chromosome and chromatid breaks, sister chromatid aberrations, somatic crossing over, nondisjuction, sister chromatid exchange, and micronuclei are available in plant test systems. Perhaps the greatest advantage plants offer over other test systems is the ease with which genotoxicity in somatic and germ cells can be measured in the same organism. In a number of plant species (e.g., Vicia faba, Trandescantia nadulans Zea mays, and Hordeum vulgare), chromosomal aberrations may be measured in the root apex and gene mutation and chromosomal aberration in the pollen mother cells. In certain plant test systems such as Hordeum and Arabidopsis, the genetic end point monitored is controlled by several hundred loci distributed throughout the genome. Therefore, mutagenic response is not limited to a single locus; instead, a large number of loci are simultaneously monitored.

Reliability

Many genetic end points reflecting mutational events in plants are very easily scored. One example is the genetic changes causing color changes in the entire plant or parts of the plant such as leaf spots and stamen hair cells. Several simple tests have also been developed to indicate mutagenesis in less easily observed cases. For example, the iodine test may be used to distinguish starchy from waxy pollen in maize and therefore detect forward or reverse mutation. Thus, in terms of minimizing observer bias, plants are ideal test systems.

Another criterion of reliability relates to the potential of plant test systems to predict mammalian mutagenic effects. Several studies have shown excellent qualitative correlations between the response of plant and mammalian test systems (Vig, 1978). Grant (1978) has listed the results of a study of a number of pesticides in which clastogenic response in plant root tips and mutagenic response in mammalian cell cultures were compared.

These results are presented in Table 1. Parallel response of these pesticide chemicals in plant test and mammalian cell culture bioassays is evident from this table. 5

Table 1. Comparison Between Chromosome Breaking and Mutagenic Effects of Pesticides in Plant and Animal Materials[a]

Compound	Chromosome Aberrations		Mutagenic Effect
	Plant Root Tips	Cells in Culture	
Apholate	+	+	?
Atrazine	+	+	+
2,4-D	+	+	±
DDT	+	+	+
Dichlorvos	+	+	+
Dieldrin	+	+	±
Ethylene dibromide	-	-	+
Griseofulvin	+	+	-
Hempa	-	-	+
Heptachlor	+	+	+
Maleic hydrazide	+	-	+
Mercury compounds	+	+	?
Phosphamidon	+	+	?
2,4,5-T	+	+	±
Tepa	+	+	+

[a]Data from Grant (1978).

In a recent study, Grant et al. (in press) have compared the clastogenic response in plant test systems with the mutagenic response of eight pesticides in a battery of microbial and mammalian cell bioassays. A summary of the data is shown in Table 2. An excellent concordance between the results from a Vicia faba bioassay and mutagenic response in other bioassays is evident from the table.

While these findings clearly indicate the general utility of plant test systems, it should be pointed out that exceptions to this concordance do exist. Thus far, the effects of daunomycin,

adriamycin, anthracycline, cytosine arabinoside, and maleic hydrazide have been demonstrated to be different in plant and animal test systems (Nilan and Vig, 1976; Vig, 1978).

Table 2. Comparison of Genetic Response of Certain Pesticides in a Battery of Microbial and Mammalian Cells and in *Vicia faba*[a]

	Vicia faba	Microbes			Mammalian Cells		
Pesticide	Chromosomal Aberrations	*S. typ.*	*S. cerev.* D3	*S. cerev.* D7	CHO (SCE)	L5178Y $TK^{+/-}$	WI38 UDS
Acephate	+	+	+	+	+	+	+
Bromacil	+	o	o	o	+	+	o
Captan	+	+	+	+	o	+	-
Demeton	+	+	+	+	+	+	+
Folpet	+	+	+	o	o	+	-
Monocrotophos	+	+	+	+	+	+	+
Parathion-methyl	+	-	+	-	+	+	-
Trichlorfon	+	+	+	+	+	+	+

[a]Unpublished data.
+ = Positive.
- = Negative.
o = Not tested.

Another question that arises relates to how plant test systems compare with other test systems in suggesting the potency of various mutagenic chemicals. Clive (1981) has surveyed the literature and made comparisons of the response of various test systems to chemical mutagens. The results are shown in Table 3. The chemicals were ethyl methanesulfonate (EMS), methyl methanesulfonate (MMS), ethyle imine (EI), N-methyl-n'-nitro-N-nitrosoguanidine (MNNG), dimethylnitrosamine (DMN), triethylenemelamine (TEM), trenimon (TREN), and mitomycin C (MMC). Results of these rankings indicate that the response of plant test systems to these chemicals was very similar to the response in *in vitro* mammalian test systems. In fact, the responses of the plant systems appear to more closely resemble that of the two mammalian systems than that of *Drosophila* or bacteria. Since these results represent only a handful of chemicals, further validation is

Table 3. Ranking of Mutagenic Potencies (Frequency/Dose) of Eight Chemical Mutagens for Plants and Other Systems[a,b]

Plants	Mammals			
In Vivo	In Vivo	In Vitro	Drosophila	Bacteria
TREN	TEM	TREN	TEM	MMC
MMC	TREN	MMC	TREN	TEM
MNNG	MMC	EI	MMS	EI
TEM	EI	MNNG	DMN	DMN
EI	DMN	TEM	MNNG	MNNG
EMS	MNNG	MMS	EMS	MMS
MMS	MMS	DMN	MMC	TREN
DMN	EMS	EMS	EI	EMS

[a]Clive 1981.
[b]Chemicals are ranked in order of decreasing potency in each system.

needed for using plant test systems for ranking the relative mutagenic potency of environmental chemicals. However, these findings examined as a whole indicate that plant systems show great promise as reliable mutagenesis screens and monitors.

Test Feasibility

As environmental monitors and screens, green plants probably represent one of the most inexpensive, reliable systems available. Most plants are easily grown and, in cases where seeds or cuttings are used, little laboratory space is required. The training of technicians also is accomplished easily. Elaborate protocols for aseptic culture are usually unnecessary. As monitors of pollutants, plants are unique in their applicability to the problem of *in situ* pollutant monitoring of both terrestrial and aquatic habitats. In this area of research, plant test systems may be used for short-term monitoring studies to assess continuing, low-level pollution effects and to monitor complex mixtures of pollutants -- mixtures that may act synergistically to increase the mutagenic potencies. In these uses, plant systems are thus far the only test systems with general utility.

In the ambient environment, the observed effects may be produced as a result of multimedia exposure, (i.e., air, water, and soil). In the laboratory, the test plants may be raised either in growth chambers or greenhouses and treated with the test compound by fumigation, irrigation, spraying, growing plant cuttings in liquid medium, or for chronic studies, growing plants in soil containing test substances.

PLANT TEST SYSTEMS

As mentioned earlier, several reviews have recently appeared on the utility of plant test systems for monitoring mutagens in the environment. In this review, only a brief description of selected test systems is presented. For discussion, plant bioassays are organized into cytogenetic and specific locus test systems. Cytogenetic test systems are more commonly employed in the laboratory to evaluate the clastogenic potential of environmental substances, whereas specific locus tests are more commonly used to evaluate the mutagenicity of the total environment (i.e., in situ monitoring). However, either of the test systems could be used for in situ monitoring or laboratory screening. Furthermore, the same indicator organism (e.g., Tradescantia, Hordeum, or Zea) may be used for chromosomal aberrations as well as for specific gene mutations.

Cytogenetic Test Systems

Somatic tissue, usually root tip meristematic cells, or germinal tissue such as pollen mother cells may be used to determine a substance's ability to cause chromosomal aberrations. The assays using root tip mitotic cells for chromosomal aberration are discussed first, followed by those assayed using germ cells.

Plant root tips as a test system. Root tips are suitable tissue for studying the effects of chemicals on chromosomes (Khilman, 1971). Root tips are relatively inexpensive to obtain in large numbers, and they handle easily and display a wide range of sensitivity to low chemical concentrations. The species most commonly used for this purpose are broad beans (Vicia faba), onions (Allium cepa), barley (Hordeum vulgare), corn (Zea mays), and Tradescantia paludosa. These species offer the advantages of having a small number of large-sized chromosomes. Although protocols for cytogenetic evaluation of each of these test systems vary because of differences in growth requirements of the plant species, duration of the mitotic cycle, and period of DNA synthesis, the principles these test systems are based on are similar. The general procedure is to suspend the young, growing

roots, 2 to 3 cm in length, in appropriate concentrations of test solution for the duration of 2 to 24 h, followed by a recovery period of 4 to 72 h. The recovery period before fixation is needed to allow the damaged chromosomes to arrive at the metaphase. The roots are excised and treated with colchicine (0.05 to 0.2%) or with 0.002 M 8-hydroxyquinoline to obtain large numbers of metaphase chromosomes. This procedure is followed by fixation, staining, maceration, and slide preparation. Both chromosomal and chromatid aberrations should be scored.

Both negative and positive controls must be included in the experiment. The most commonly used positive controls are ethylmethane sulfonate (EMS), and X rays. The negative controls for non-water-soluble chemicals may be dimethyl sulfoxide (DMSO), ethanol, or weak acids. The experimental data may be greatly affected by variations in pH and temperature. For details on the protocols for each test system, Ma (in press a,b), Khilman (1971, 1975), Wolff (1964), Grant (in press), Auerbach (1976), Constantin and Nilan (1982), and Sharma and Sharma (1972) may be consulted.

Pollen mother cells (PMC) as a test system. Although any plant species with a relatively small number of large-sized chromosomes may be used for meiotic chromosomal aberrations, only three species have been commonly used. These are Tradescantia paludosa (2n = 12), Vicia faba (2n = 12), and Hordeum vulgare (2n = 14). The use of PMC in cytogenetics offers the advantage of evaluating potential heritability of the genetic lesions. Except for the Tradescantia micronucleus test where the plant cuttings are exposed to the test substance, the preferred method is to expose presoaked seeds to the test substance. Usually the chromosomal aberrations are evaluated in the roots of the young seedlings, and after numerous cell divisions, metabolic activation, and exposure to environmental dynamics, cytogenetic effects are reevaluated in the germ cells. Thus, the integrated results of cytogenetics from the somatic and germ cells provide a better means of evaluating the genetic hazard of a chemical than the results from either tissue alone. Experimental procedures for PMC cytogenetics for Vicia, Tradescantia (Ma, in press a,b) and Hordeum (Constantin and Nilan, 1982) will be published soon in Mutation Research. Vicia and Hordeum meiotic tests measure the entire spectrum of chromosomal aberrations, whereas the Tradescantia test has been designed to evaluate only the micronuclei that may be formed by acentric fragments or sticky chromosomes. The Tradescantia micronucleus test (Trad-MCN), developed by Ma of Western Illinois University, is the simplest and perhaps the most sensitive of all the plant cytogenetic assays currently used. Young inflorescences of plant cuttings are exposed to test agents either through absorption of liquid medium

or diffusion from the gaseous substances. The micronuclei are scored in the tetrad 24 h after the exposure. This assay has been used for evaluating the mutagenicity of gases, liquids, and chemical agents and for *in situ* monitoring (Ma et al., 1982).

Specific Locus Test Systems

As in the case of chromosomal aberrations, point mutations in plants could be detected either in somatic cells or in germ cells. The most commonly used assays for detecting environmental mutagens in plant somatic cells are the *Tradescantia* stamen hair assay, the *Arabidopsis* multilocus assay, the *Hordeum* chlorophyll deficiency assay, and the *Glycine* leaf spot assay. The most often used germ cell gene mutation assays in plants utilize waxy locus in cereals (especially maize and barley) and adh locus in maize.

Somatic cell assays. The *Tradescantia* stamen hair assay, originally developed by Sparrow (Underbrink et al., 1973a), is the most frequently used plant bioassay for monitoring atmospheric pollutants in the United States (Schairer et al., 1978) and Japan (Ichikawa, 1981). This assay is based on the detection of changes in the color of stamen hair cells from blue (dominant) to pink (recessive). The assay uses Clone 4430, an interspecific hybrid between *T. hirsutiflora* (homozygous blue) and *T. subacaulis* (homozygous pink) for flower color (Van't Hof and Schairer, in press). The experimental procedures and genetic basis for the test have been described by Underbrink et al. (1973b), Sparrow et al. (1974), Schairer et al. (1978), and Thompson-Emmerling and Narwrocky (1980) The reliability of this assay system has been adequately evaluated for chemical mutagens in gaseous and liquid forms (Van't Hof and Schairer, in press) and for radiation (Ichikawa, 1981).

For a mutagenicity assay to be performed in the laboratory, *Tradescantia* cuttings bearing inflorescences are exposed to the test substance for a few hours to several days. After treatment, cuttings are kept in a nutrient solution until the observations have been completed. Each inflorescence contains approximately 20 flower buds arranged in various developmental stages. Each stamen hair is composed of approximately 25 cells. The mutated cells (pink) are easily distinguishable from normal cells (blue) under a wide field microscope.

Recently the *Tradescantia* stamen hair assay has been used to monitor the ambient air quality of several cities in the United States for gaseous air pollutants (Schairer et al., 1982) and for detecting levels of radiation around nuclear power plants in Japan (Ichikawa, 1981). Under field conditions, plants are exposed to

ambient air in a specially constructed mobile van for a period of 17 days. Concurrent-negative controls are included in the protocol. The results of ambient air monitoring for one of the studies conducted in the United States are shown in Figure 1. This figure shows the feasibility of ranking ambient air quality in various cities using the Tradescantia stamen hair test system. For example, Elizabeth, New Jersey, which has a high concentration of the petrochemical industry, had the highest elevation of Tradescantia pink mutants. Similarly, studies conducted with Tradescantia by Ichikawa (1981) around nuclear power plants in Japan showed significantly increased mutation frequencies around these plants. The elevated pink mutation frequencies were correlated with the operation periods of the nuclear facilities and the predominant wind direction.

This assay is very sensitive and offers the opportunity to simultaneously monitor gene mutations and chromosomal aberrations. However, counting 300,000 to 400,000 stamen hairs for each treatment is laborious and limits the use of this assay system. In addition, the nature of the mutant events and chemistry of the flower pigmentation need to be further studied.

Arabidopsis multilocus assay. Arabidopsis is a short cruciferous plant that can complete its life cycle in four to six weeks. Thousands of plants can be grown in a small growth chamber in the laboratory, greenhouse, or field under diverse climatic conditions. A single plant can produce as many as 5000 seeds. This assay is capable of simultaneously detecting mutation at thousands of loci (Redei, in press). The test can be performed by either observing segregating M_2 embryos in the fruits of M_1 plants before they mature or by growing M_2 progeny. The former method is preferred because it is faster and more efficient.

The forward mutation test system was first reported by Miller and further developed by Redei (in press). The assay consists of exposing mature seeds to the mutagens and analyzing the chlorophyll-deficient M_2 embryos on the M_1 plants. The rationale, experimental procedure, and statistical analysis pertinent to this assay have been discussed by Redei (in press). A fairly high correlation (around 90%) between mutagenicity in Arabidopsis and carcinogenicity in animals has been observed by Redei et al. (1980) for diverse classes of chemicals. A number of nitroso compounds that require metabolic activation to produce genetic lesions were also genetically reactive in Arabidopsis. Because of the short life cycle, small size, high fecundity, and wide adaptability, this bioassay seems to be suitable for screening the

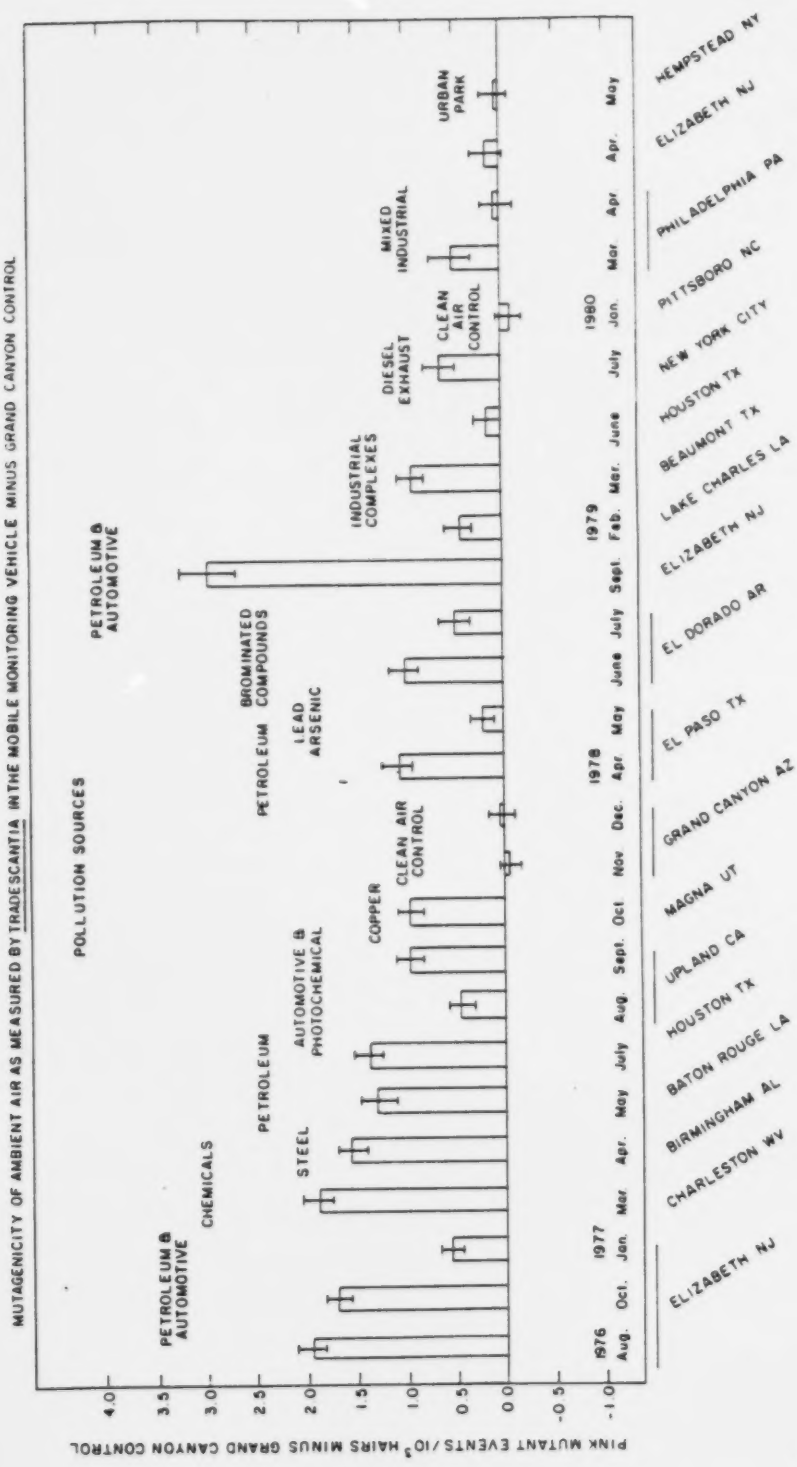

Fig. 1. Ranking of ambient air quality in various U.S. cities.

potential genetic damage from exposure to environmental substances. However, the performance of this bioassay for *in situ* monitoring has not been adequately evaluated.

Hordeum chlorphyll-deficient mutant assay. Historically, barley has played a significant role in developing the principles of genetics and illustrating the basis for induced mutations. This bioassay consists of exposing seeds or seedlings to test chemicals and observing chlorophyll-deficient mutants in the following generation. These mutant phenotypes are controlled by hundreds of recessively inherited genes that are distributed throughout the genome. The genetic basis for this assay along with experimental procedure and statistical methodology has been described by Constantin and Nilan (1982).

Glycine leaf spot assay. Developed by B. K. Vig of the University of Nevada, this test system is capable of simultaneously monitoring somatic crossing over, chromosomal loss or gain, and gene mutation. The test system has the advantages of providing resolution of the mechanism underlying genetic damage and being rapid (four to five weeks) and relatively inexpensive. The system has been validated with a variety of test compounds and compares favorably with *in vitro* mammalian cell systems. The advantage over the mammalian test is that it is less expensive and has an intrinsic metabolic activation capability.

Briefly, varieties L65-1237 or T219 with genotype Y11 y11 and light-green phenotype are used as a tester strain. Four to five weeks after the seeds have been treated with the test compound, the distribution of spots appearing through the leaves is analyzed as follows: the appearance of dark green and yellow spots indicates somatic crossing over, a single spot reflects the gain or loss of a chromosome, and the increase in frequency of light-green spots on golden yellow leaves indicates back mutation. The genetic basis and experimental protocol for this test system has been reviewed by Vig (in press). Although this test system offers the opportunity of simultaneously testing the chromosomal effects and point mutations, it needs further validation with large numbers of chemicals. Also the nature of genetic events needs to be confirmed.

Germinal cell mutagenesis. The use of pollen grains for mutagenesis offers unique advantages: 1) The unit of measurement is a pollen grain, and millions are available; thus, the resolving power of the test system is equivalent to microbial test systems, but it retains the advantages of eukaryotic organization. 2) The end points are a specific gene product. 3) The functional unit is a haploid germinal cell indicating potential of heritable damage.

As with the use of plant somatic cells, pollen grains of any species could be used for mutagenesis studies. However waxy and adh locus systems in maize warrant special attention. These assays have been selected here for a brief description because the nature of the altered gene product is better understood, and the end points for these assays are identified quite easily.

Maize waxy locus assay. The historical background, genetic basis, and assay protocol for this test have been presented by Plewa (1978, 1981, and in press). Briefly, the plants homozygous for waxy alleles (wx/wx) produce endosperm that contain only amylopectin, and the plants homozygous (Wx/Wx) or heterozygous (Wx/wx) for sugar alleles produce starch in the endosperm containing amylopectin and amylose. Since amylose stains dark blue-black when it reacts with iodine, and amylopectin does not react with iodine (endosperm stains red), these two phenotypes could be easily identified.

The starch type in a pollen grain is controlled by its own genetic constitution. Since a pollen grain is a functional unit, its genotype can be identified by the iodine test (Plewa, in press). Therefore, forward mutation (Wx → wx) can be detected by measuring fractions of pollen grains that stain reddish-tan from plants homozygous at the sugary locus (Wx/Wx). Conversely, reverse mutation can be measured by scoring the pollen grains that stain dark blue-black from light staining pollen from plants that are homozygous for waxy alleles (wx/wx).

This assay system achieved prominance in 1978 when Plewa and his associates published reports showing that maize plants grown under field conditions provide the needed metabolic activation to transform certain pesticides into mutagens (Gentile et al., 1978). This system has been used for in situ monitoring of the mutagenic compounds in municipal sludges (Hopke et al., 1982) and ambient air quality (Lower et al., 1978). Also, this test system has been employed for detecting ionizing radiation (Erikson, 1963, 1971; Erikson and Tavrin, 1965). Recently, Plewa (1980) has produced very early maturing short-statured tester lines that will enhance the utility of this test system for use in laboratory screens and for on-site monitoring.

FUTURE RESEARCH NEEDS

Although green plants offer excellent material for mutagenesis studies, their use for this purpose has been rather limited and ignored in the recent past. As a result, an intensive

research effort has to be made before plant assays can effectively be used to evaluate environmental genotoxins. Listed below are a few areas that should be emphasized:

1. The nature of the mutational events needs to be adequately defined. Pollen grains provide an excellent material for this type of study.

2. A series of multiple genetic markers should be incorporated into the same bioassay system to enhance its sensitivity and reliability.

3. The utility of certain bioassays such as Tradescantia stamen hair assay, adh maize, and waxy maize can be greatly enhanced by automation techniques. Counting the thousands of pollen grains or stamen hair cells is very tedious, costly, and time-consuming. Some progress has already been made toward achieving this goal (Amano, 1981; Pinkel 1981).

4. The plant bioassays need further validation for sensitivity and reliability. Dose-response curves for plant test systems need to be established with large numbers of chemicals. A comparison of mutagenic response of chemicals in animal and plant test systems should be made.

5. The statistical models for the analysis and interpretation of the plant test data are needed.

6. The mitotic and meiotic end points should be integrated and evaluated.

7. Early maturing miniaturized plants should be produced in order to reduce the study time and cost.

8. Repair-sensitive strains should be identified.

9. Environmental variables such as pH, temperature, photoperiod, and humidity as they affect mutagenic response for each test system should be identified.

10. Plant in vitro bioassays need to be developed and validated.

PLANTS AS A PART OF THE MUTAGEN TEST SCHEME

Plants can play an important role in the phased testing scheme proposed by Waters (1980) for evaluating genotoxicity and presumptive carcinogenicity of environmental chemicals. The root tip bioassays fulfill all the conditions necessary for

incorporation into level I. These conditions are simplicity, low cost, short study time, reliability, and sensitivity. The point mutation bioassays using somatic or germinal cells may be included in the level II test battery for mutagenicity evaluation. Because of the differences in metabolism in plants and animals as well as tissue and organ specificity of certain classes of chemicals, the mutagenicity data from plant test systems may be considered only in a qualitative sense. However, since plants are closer to animals in terms of genetic organization and evolutionary divergence, mutational data from plant systems should be assigned greater significance than similar data from microbial test systems.

REFERENCES

Amano, E., 1981, Flow system for automated analysis of maize pollen Environ. Hlth. Perspect., 37:165.

Auerbach, C., 1976, "Mutation Research: Problems, Results, and Perspectives," Chapman and Hall, London.

Clive, D., 1981, Comparative mutagenicity, in: "Comparative Mutagenesis: Can We Make Risk Estimations?," F. J. Serres and M. D. Shelby, eds., Plenum Press, New York.

Constantin, M. J., 1978, Utility of specific locus systems in higher plants to monitor for mutagens, Environ. Hlth. Perspect 27:69.

Constantin, M. J., and Nilan, R., 1982, Chromosome aberration assays in barley (Hordeum vulgare), Mutation Res., 99:13.

Erikson, G., 1963, Induction of waxy mutants in maize by acute and chronic gamma irradiation, Hereditas, 50:161.

Erikson, G., 1971, Variation in radiosensitivity and dose effect relationships in low scope range, Hereditas, 68:101.

Erikson, G., and Tavrin, E., 1965, Variations in radiosensitivity during meiosis of pollen mother cells in maize, Hereditas, 54:156.

Feder, W. A., 1978, Plants as bioassay systems for monitoring atmospheric pollutants, Environ. Hlth. Perspect., 27:139.

Freeling, M., 1978, Maize adhl as monitor of environmental mutagens, Environ. Hlth. Perspect., 27:91.

Gentile, J. M., Wagner, E. D., and Plewa, M. J., 1978, A comprehensive analysis of the mutagenic properties of pesticides used in commercial maize production, Mutation Res., 53:112.

Grant, W. F., 1978, Chromosome aberrations in plants as a monitoring system, Environ. Hlth. Perspect., 27:37.

Grant, W. F., 1981, Plant mutagen assays based upon chromosomal mutations, in: "Environmental Mutagenesis, Carcinogenesis and Plant Biology," E. Klekowski, ed., Praeger, New York.

Grant, W. F., in press, Chromosome aberrations assay in Allium, A report of the U.S. Environmental Protection Agency GENE-TOX Program, Mutation Res.

Grant, W. F., and Zura, K. V., in press, Plants as sensitive in situ detectors of atmospheric mutagens, in: "Mutagenicity: New Horizons in Toxicology," J. A. Heddle, ed., Academic

Green, S., and Auletta, A., 1980, Editorial introduction to the reports of "The GENE-TOX Program," Mutation Res., 76:165.

Hopke, P. K., Plewa, M. J., Johnston, J. B., Weaver, D., Wood, S. G., Larson, R. A., and Hinesly, T., 1982, Multitechnique screening of Chicago municiple sewage style for mutagenic activity, Environ. Sci. Technol., 16:140.

Ichikawa, S., 1981, In situ monitoring with Tradescantia around nuclear power plants, Environ. Hlth. Perspect., 37:145.

Kihlman, B. A., 1966, "Actions of Chemicals on Dividing Cells," Prentice Hall, Inc., Englewood Cliff, NJ.

Kihlman, B. A., 1971, Root tips for studying the effects of chemicals on chromosomes, in: "Chemical Mutagens: Principles and Methods for Their Detection," vol. 2, A. Holleander, ed., Plenum Press, New York.

Kihlman, B. A. 1975, Root tips of Vicia faba for the study of the induction of chromosomal aberrations, Mutation Res., 31:401.

Lower, W. R., Rose, P. S., Drobney, V. K., 1978, In situ mutagenic and other effects associated with lead smelting, Mutation Res., 54:83.

Ma, T. H, in press, Vicia cytogenetic tests for environmental mutagens, A report of the U.S. Environmental Protection Agency GENE-TOX Program, Mutation Res. (a)

Ma, T. H., in press, Tradescantia cytogenetic tests for environmental mutagens, A report of the U.S. Environmental Protection Agency GENE-TOX Program, Mutation Res. (b)

Ma, T. H., Fang, T., Ho, J., Chen, D., Zhou, R., Lin, G., Dai, J., and Li, J., 1982, Hypersensitivity of Tradescantia reflexa meiotic chromosomes to mutagens, Mutation Res., 104:101.

Maugh, T. H., 1978, Chemicals: how many are there?, Science, 199:162.

Menn, J. J., 1978, Comparative effects of pesticide metabolism in plants and animals, Environ. Hlth. Perspect., 27:113.

Nilan, R. A., 1978, Potential of plant genetic systems for monitoring and screening mutagens, Environ. Hlth. Perspect., 27:181.

Nilan, R. A., and Vig, B., 1976, Plant test systems for detection of chemical mutagens, in: "Chemical Mutagens: Principles and Methods of Their Detection," vol. 4, A. Holleander, ed., Plenum Press, New York.

Pinkel, D., 1981, On the possibility of automated scoring of pollen mutants, Environ. Hlth. Perspect., 37:133.

Plewa, M. J., 1978, Activation of chemicals into mutagens by green plants: A preliminary discussion, Environ. Hlth. Perspect., 27:45.

Plewa, M. J., 1980, Forward mutation in inbred early synthetic Zea maize by chronic exposure to EMS, Environ. Mutagen., 2:286.

Plewa, M. J., and Wagner, E. D., 1981, Germinal cell mutagenesis in specially designed maize genotypes, Environ. Hlth. Perspect., 37:61.

Plewa, M. J., in press, Specific locus mutation assay in Zea maize, A report of the U.S. Environmental Protection Agency GENE-TOX Program, Mutation Res.

Plewa, M. J., and Gentile, J. M., 1981, The activation of chemical mutagens by green plants, in: "Chemical Mutagens: Principles and Methods for Their Dectection," vol. 7, A. Holleander and F. J. de Serres, eds. Plenum Press, New York.

Plewa, M. J., Wagner, E. D., and Gentile, J. M., 1979, An analysis of the mutagenic properties of pesticides incorporating animal and plant activation, Environ. Mutagen., 1:142.

Redei, G. P., in press, Arabidopsis assay of environmental mutagens, A report of the U.S. Environmental Protection Agency GENE-TOX Program, Mutation Res.

Redei, G. P., Redei, M. M., Lower, W. R., and Sandhu, S. S., 1980, Identification of carcinogens by mutagenicity for Arabidopsis, Mutation Res., 74:469.

Schairer, L. A., Van't Hof, J., Hays, C. G., Burton, R. M., and de Serres, F. J., 1978, Exploratory monitoring of air pollutants for mutagenicity activity with Tradescantia stamen hair system, Environ. Hlth. Perspect., 7:151.

Schairer, L. A., Santkulis, R. C., and Tempel, N. R., 1982, A search for the identity of genotoxic agents in the ambient air using the Tradescantia bioassay, in: "Application of Short-Term Bioassays in the Analysis of Complex Environmental Mixtures III," M. D. Waters, S. S. Sandhu, J. Lewtas, L. Claxton, N. Chernoff, and S. Nesnow, eds., Plenum Press, New York.

Sharma, A. K., and Sharma, A., 1972, "Chromosome Techniques, Theory and Practice," University Press, Baltimore.

Sparrow, A. H., Schairer, L. A., and Villalohos-Pietrini, R., 1974, Comparison of somatic mutation rates induced in Tradescantia by chemical and physical mutagens, Mutation Res., 26:265.

Thompson-Emmerling, M., and Narwrocky, M., 1980, Genetic basis for using Tradescantia Clone 4430 as sensor of environmental mutagens, J. Heredity, 71:261.

Underbrink, A. G., Schairer, L. A., and Sparrow, A. H., 1973a, The biophysical properties of 3.9 GeV nitrogen ions, V, Determination of the relative biological effectiveness for somatic mutations in Tradescantia, Radiat. Res., 55:437.

Underbrink, A. G., Schairer, L. A., and Sparrow, A. H., 1973b, Tradescantia stamen hair: A radiobiological test system applicable to chemical mutagenesis, in: "Chemical Mutagens: Principles and Methods of Their Detection," vol. 3, A. Holleander, ed., Plenum Press, New York.

Van't Hof, J., and Schairer, L. A., in press, Tradescantia assay system for gaseous mutagens, A report of the U.S. Environmental Protection Agency GENE-TOX Program, Mutation Res.

Vig, B., 1978, Somatic nosaicism in plants with special reference to somatic crossing over, Environ. Hlth. Perspect., 27:27.

Vig, B., in press, Soybean (Glycine max (L.) Merrill) as a short-term assay for study of environmental mutagens, A report of the U.S. Environmental Protection Agency GENE-TOX Program, Mutation Res.

Waters, M. D., Simmon, V. F., Mitchell, A. D., Jorgenson, T. A., and Valencia, R., 1980, An overview of mutagenicity/carcinogenicity research on pesticide chemicals, J. Environ. Sci. Hlth., B15:867.

Wolff, S., 1964, Handling of root tips, in: "Methods in Cell Physiology, D. M. Prescott, ed., Academic Press, New York.